BIOCHEMICAL GENETICS[1]

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The field under review is growing so rapidly that it is impossible to cover more than a sampling of recent papers in the allotted space. Important subjects such as the genetics and chemistry of viruses and certain topics in bacterial genetics have had to be omitted, while others have not received the treatment they deserve. Studies of a primarily biochemical nature in which mutants have been employed as tools have been reviewed, as is customary, although it is recognized that their genetic interest lies chiefly in their providing materials for the further study of gene action.

Chemistry of the Nucleus

Two symposia on nucleic acids (1, 2) should be referred to for a wealth of details and discussion. Here an attempt will be made to summarize pertinent data regarding the composition of cell nuclei and chromosomes.

Following Crossman (3), who introduced the use of citric acid as a fixative prior to isolation of cell nuclei, Mirsky & Ris (4), Vendrely & Vendrely (5), Boivin *et al.* (6), Heath (7), and others have investigated the composition of purified nuclei. Evidence that these preparations contain very little cytoplasmic material appears to be unquestioned. On the other hand, the isolation of chromosomes from numerous tissues, as reported by Mirsky & Ris (8, 9), has been seriously questioned by Lamb (10). The latter author states that "the threads are not isolated chromosomes but are complex fragments of drawn-out nuclei." In any case, the composition of the isolated chromosomes of Mirsky & Ris appears at least qualitatively the same as that of whole nuclei and that of chromosomes investigated by other means of isolation. The molecular species agreed upon by various workers are desoxyribonucleoproteins, ribonucleic acid (RNA), and "residual protein." According to Vendrely & Vendrely as well as Mirsky & Ris, desoxyribonucleic acid (DNA) is present in a constant quantity in the nuclei of different kinds of cells of the same species with the exception of sperm which contain half the quantity of DNA found in other tissues of the species. Since other nuclear components vary widely with types of cells within a species, these authors conclude that DNA is of primary significance in genic material. That the required specificity may, indeed, reside in nucleic acid molecules seems indicated from the work on transforming principles in bacteria.

With regard to the "residual" protein of nuclei, Mirsky & Ris (9) report that it varies in quantity directly as the metabolic activity of the cell and as the proportion of cytoplasm to nucleus. This appears to give it a functional

[1] This review covers the period September, 1947 to September, 1950.

relation to biochemical activities of the cytoplasm. It remains as a fiber after dissolving away the DNA and histone and, thus, may make up the thread of the chromosome fundamental to the linear order of genes. Amino acid composition of residual protein has been investigated by Blumel & Kirby (11), who report the presence of aspartic and glutamic acids, glycine, valine, alanine, leucine, and proline. Tryptophan would be destroyed by the hydrolysis conditions used, but other workers have reported its presence.

Kaufmann *et al.* (12) and, more recently, Mazia (13) have reported some informative experiments on the action of highly purified enzymes on nuclear components. The former, using sectional material from lily and onion root tips as well as salivary gland chromosomes from *Drosophila,* combined enzymatic digestions with specific staining techniques to provide evidence that the chromosomes contain both DNA and RNA while the nucleolus and cytoplasm contains only RNA. Mazia (13), using somewhat similar techniques, has provided evidence that salivary chromosomes contain continuous threads of both histonelike protein (carrying DNA) and an acidic residual protein. Digestion with pepsin, which attacks the residual protein but not histone, causes shrinkage of the chromosomes, an effect which is greater between the prominent nucleic acid bands than in the bands themselves. Further experiments of Mazia have called attention to the fact that cathepsin and crude preparations of proteolytic enzymes hydrolyze thymus nucleohistone but do not attack salivary chromosomes. It is concluded that this lack of action is due to the physical state rather than the chemical composition of the chromosomes. Some interesting model systems are described.

In view of the present trend toward considering DNA to carry genic specificities, the properties of these substances have become of particular interest. Butler *et al.* (14) have compared the effects of x-rays with those of sulfur and nitrogen mustards on the "structural viscosity" of highly polymerized thymus DNA. These treatments produced similar effects in reduction of viscosity at a slow rate of flow, but in addition, it was observed that decrease in viscosity continued apparently for many hours after cessation of x-radiation. Such an effect is reminiscent of the "delayed" mutation in bacteria observed by Demerec & Latarjet (15). The experiments of Scholes *et al.* (16) on high dosage irradiations (x-rays) of DNA and RNA are of considerable interest in that they indicate the kinds of chemical changes that take place in the nucleic acids as a result of x-irradiation. It is reported that (*a*) glycosidic linkages are broken; (*b*) deamination of bases occurs; (*c*) heterocyclic rings are broken; and (*d*) ester linkages are broken, releasing primary and secondary phosphates. More recently, Rapport & Canzanelli (17) have investigated the effects of ultraviolet radiation on nucleic acids with a demonstration of almost complete loss of absorption spectra and, thus, a probable breakage of heterocyclic rings. Even though the dosages used in these experiments were high, a single change of any of the types mentioned may be sufficient to alter the specific structure and function of a macromolecule of nucleic acid. Thus, it seems probable that mutation can

be accounted for on the basis of one or more of a wide variety of chemical changes.

Investigations by Ris & Mirsky (18) have produced convincing evidence that chromosomes of interphase nuclei have not lost their continuity but have become invisible by usual techniques of observation through an enormous swelling of the DNA component. Reversible swelling and condensation, resulting from treatments with sugar and salt solutions respectively, was demonstrated along with techniques for fixing in either state. It should be of great interest to determine whether or not this change in state is directly related to the gene functions of selfreproduction and control of metabolic processes.

Mutagenic Agents

Chemical mutagens.—The discovery by Auerbach & Robson that mustard gas induces mutations in *Drosophila* [discussed by Beadle (19)] has reawakened interest in the search for chemical mutagens [reviewed by Auerbach (20)]. It is hoped, through the study of such reagents, to arrive at deductions concerning the functional groups of genes and the chemical nature of the mutational process. A feature of recent work has been the large number of reports of mutagenic effects with chemicals, in contrast to the almost totally negative results of the preceding 25 years. This is due to the impetus given this field by the mustard gas discovery and to the use of improved methods for the detection of mutants. By determining mutations to drug or phage resistance in bacteria, for example, or back-mutations of biochemical mutants in *Neurospora* (21, 22), conditions can be arranged so that only mutants (or back-mutants) will grow on a plate. With the background of unaffected cells thus eliminated, enormous numbers of cells can be readily tested and even weak mutagenic activities detected. A danger in such experiments is the possibility of confusing mutagenicity with selective action of the agent in favor of spontaneous mutants. This is especially true if growth is permitted to take place in the presence of the agent. A simplified method for applying the Luria-Delbrück fluctuation test (23) to such cases has been devised by Newcombe (24).

Among compounds other than mustards recently reported to produce gene mutations are organic peroxides [Dickey *et al.* (25)], hydrogen peroxide and cyanide [Wagner *et al.* (26)], caffeine and theophylline [Fries & Kihlman (27, 28, 29)], diazomethane [Jensen *et al.* (30)], and urethane [Vogt (31)]. In addition, the following substances have been found by cytological examination to cause chromosomal damage and may be regarded as potential mutagens: di-epoxides [Loveless & Revell (32), Ross (33)], *o*-isopropyl-N-phenylcarbamate [Ennis (34)], hexachlorocyclohexane [Kostov (35)], and acriflavine [Bauch (36)]. Acriflavine, desoxycholate, and pyronin increase the frequency of phage-resistant variants in nongrowing suspensions of *Escherichia coli*, probably as a result of mutagenic action [Witkin (37)].

The demonstration of a mutagenic effect does not necessarily imply a direct reaction of the agent with the gene. It is probable that in many cases the effect is mediated by substances produced in the cytoplasm. It has been

proposed that free radicals play an essential part in the mutagenic effects of peroxides (25), diazomethane (30), and other agents. Cyanide may act by poisoning catalase, thus allowing peroxides to accumulate in the cell (26). Indirect action is highly probable in the case of formaldehyde which was first shown by Rapoport (38) to induce mutations when fed to *Drosophila*. The effect has been confirmed by several investigators who find that formaldehyde is active when mixed with the food [Kaplan (39), Auerbach (40)], but not when applied as a vapor (40) or in a vaginal douche [Herskowitz (41)]. It is inferred that the actual mutagen is produced by a reaction of formaldehyde with a constituent of the food (40).

The mutagenic status of the carcinogenic hydrocarbons is not clear. Demerec (42) finds that 1,2,5,6-dibenzanthracene, applied in an aerosol, induces sex-linked lethals and chromosomal aberrations in *Drosophila*. This is in contrast to earlier results of Auerbach (43), who observed no increase in frequency of sex-linked lethals following the injection of 1,2,5,6-dibenzanthracene, 9,10-dimethyl-1,2-benzanthracene, or methylcholanthrene into *Drosophila*. Methylcholanthrene has given negative or doubtful results in the hands of all investigators except Strong (44), who reports mutations in mice following subcutaneous injection of this carcinogen. Negative results were obtained by Bhattacharya (45) in feeding-experiments with *Drosophila*, and by Latarjet (46) in *E. coli*, while a doubtful effect was found by Tatum *et al.* (47) in *Neurospora*. One can draw the conclusion that there is no parallelism between carcinogenic activity and mutagenicity, as these are ordinarily determined. However, this does not necessarily disprove the mutational theory of the origin of cancer [for discussion see (20)].

The activity of the mustards is generally believed to be based on their ability to alkylate the functional groups of biological substances. Since the mustards typically contain two reactive side-chains, there is a possibility of forming cross-linkages between cellular constituents. This mechanism has been emphasized by Goldacre *et al.* (48) and by Loveless & Revell (32), based on their studies of a series of aromatic nitrogen mustards [Bird (49)] and di-epoxides. The latter are esterifying agents used industrially to bring about cross-linking between the carboxyl groups of wool fibers. They are reported to show the same cytological effects as the nitrogen mustards, producing breaks in or near heterochromatic regions in *Vicia faba* chromosomes (32, 33). It is suggested that mustards and di-epoxides act by forming cross-links between adjacent chromatid fibers.[2] Ross (33) states that organic peroxides also induce breaks in heterochromatic regions, and he presents a theory of the action of mustards, peroxides, and di-epoxides.

The genetic and cytological effects of the assimilation of P^{32} by various organisms have been investigated by Giles and co-workers (50, 51), Arnason *et al.* (52, 53), and Bateman & Sinclair (54). The effects are, in the main, indistinguishable from those of x-rays. Giles & Bolomey (50), who also

[2] Recent experiments by Stevens & Milroie (229), Auerbach & Moser (230), and Jensen *et al.* (231), however, show that two reactive groupings are not necessary for the mutagenic action of mustards.

included C^{14}. in their study, correctly emphasize the implications of these results for radioactive tracer studies on haploid microorganisms.

Radiation effects.—It is becoming increasingly clear that the genetic effects of high-energy radiation are, to an important extent, the secondary results of a radiochemical reaction in the medium or in the cellular fluids. Several lines of evidence implicate peroxide formation in the process.

Stein & Weiss (55, 56) present evidence for the idea that the primary net effect of ionizing radiations in aqueous systems is the production of hydrogen and hydroxyl radicals. De-aerated aqueous suspensions of benzene were found to contain measurable quantities of phenol and diphenyl following x-irradiation. Similarly treated solutions of benzoic acid and of amino acids yielded salicylic acid and products of oxidative deamination and decarboxylation, respectively. The authors state that similar products are formed when the mentioned substances react with chemically produced hydroxyl radicals.

Thoday & Read (57, 58), using *Vicia faba* root-tips, have found that ionizing radiations are less effective in producing chromosomal damage and growth inhibition when the irradiation is carried out anaerobically than when oxygen is present. This observation has been confirmed and extended by Giles & Riley (59, 60) on the chromosomes of *Tradescantia* microspores. These authors find as much as a fivefold increase in the frequency of chromosomal aberrations in material exposed to x-rays under oxygen as compared to material treated under nitrogen. They further show that the effect of oxygen is not on the recovery process. This makes it appear probable that the presence of oxygen increases the rate of chromosome breakage. The oxygen effect has been obtained in *Drosophila* by Baker & Sgourakis (61), who determined the rate of production of sex-linked lethals. A given dose of x-rays was approximately twice as effective when the flies were exposed in oxygen as in nitrogen. The oxygen effect is possibly related to the radiochemical production of hydrogen peroxide, since the latter is also a function of the oxygen tension (60, 62).

Along similar lines, Barrón and collaborators (63, 64) find that sulfhydryl enzymes are reversibly inactivated by x-rays when the dosage is kept below 5,000 r units. Reactivation is accomplished by the addition of glutathione. Nonsulfhydryl enzymes are considerably more resistant to irradiation. Simple thiols are also oxidized (65), some protection being afforded when catalase is present or, alternatively, when oxygen is absent. The results are interpreted on the basis of Weiss' theory of free radical formation mentioned above.

Allsopp & Catcheside (66) point out that the radiochemical theory of chromosome breakage is not inconsistent with target theory calculations unless it can be shown that radicals produced more than a few millimicrons from the target can cause the observed biological effects. At the present time the target theory provides the best quantitative account of the phenomena.

The production of mutagenic substances, probably peroxides, by ultra-

violet irradiation of broth has been investigated in a series of papers by Stone, Wyss, Haas & Clark (67 to 72). A large increase in the number of drug-resistant mutants was obtained by growing *Staphylococcus aureus* in previously irradiated media. That such media exert an inductive, and not merely a selective, action has been demonstrated (69). Wagner *et al.* (26) have found that biochemical mutations in *Neurospora* are also induced by treated broth. The mutagenic principle is fairly stable under ordinary conditions (72), but is destroyed by catalase (70). Treatment of broth with hydrogen peroxide also renders it mutagenic, but hydrogen peroxide by itself is not mutagenic for *S. aureus* (70), although it exhibits some activity in *Neurospora* (25, 26). It was suggested (70) that hydrogen peroxide formed during irradiation reacts with constituents of the medium to produce organic peroxides which are responsible for the genetic effects. This inference has been strengthened by subsequent experiments of Dickey *et al.* (25) who showed that organic peroxides are potent mutagens in *Neurospora*. It should be noted that the described effect has been observed only with wave lengths in the 2000 Å region or below (72), although it is well established that a peak of mutagenic activity occurs in the 2600 Å region when cells are irradiated directly. The latter is presumably due to absorption by nucleic acids.

Experiments on the photoreactivation of ultraviolet inactivated bacteria by Novick & Szilard (73) show that the effect of photoreactivation is to lower the effective ultraviolet dose by a constant dose-independent factor. Less extensive experiments indicate that the same rule holds in the case of ultraviolet induced mutations to phage resistance. The results can be formally interpreted on the assumption that a toxic substance is produced, a certain fraction of which is photosensitive.

Transforming principles.—Hotchkiss (74) has investigated the rate of liberation of amino acids from purified pneumococcus transforming principle, and he concludes that these originate entirely, or nearly so, from the decomposition of adenine. The protein content of the transforming principle can be, at most, 0.2 per cent and is probably less. Hotchkiss also shows that the transforming principle does not analyze as a classical polytetranucleotide.

Taylor (75) presents evidence that Type III pneumococci produce two transforming principles, one transforming rough into smooth pneumococci, the other transforming an extremely rough mutant into rough. The latter principle is also found in rough pneumococci. Taylor has also analyzed two mutants of Type III which differ morphologically from one another and from the normal and which appear to synthesize less of the type-specific polysaccharide than the normal. The transforming agents of these variants are also altered, transforming rough into the corresponding variants of Type III. Of particular interest is the observation that, acting together, the two mutant principles can transform rough into normal Type III. It is not stated whether the transforming principle of the smooth bacteria so produced is a mixture of the mutant principles or a reconstituted normal

agent. Assuming the latter to be the case leads to the possibility of intramolecular recombination between desoxyribonucleic acid molecules, perhaps similar to crossing-over within chromosomes.

Genetics and Biosynthesis

Since this subject was discussed by Beadle (19), numerous reviews have appeared elsewhere (76 to 83).

Some special techniques.—Numerous investigators have published improved methods for production and handling of biochemical mutants of various organisms. For *Neurospora*, Lein *et al.* (84) described a method for visual selection of mutants after germination of ascospores, while Tatum *et al.* (85) utilized direct plating techniques using sorbose and other substances in the culture medium to produce colonial growth. Both methods reduce the labor involved in mutant isolations at least tenfold. Fries (86) developed an efficient procedure for isolation of mutants of *Ophiostoma* based on the observation that mutants with two nutritional requirements tend to live longer under starvation conditions than certain single mutants. A similar technique was applied successfully to *Aspergillus* by Pontecorvo (87), while the same principles would seem to apply to the observation on *Achromobacter* made by Miller *et al.* (88). Davis (89, 90, 91) and Lederberg & Zinder (92) utilized differential sterilization by penicillin for increasing the efficiency of isolation of mutants of *E. coli*. In this case, it was found that growing cells are killed preferentially by penicillin, permitting a very efficient selection of nongrowing mutants. Plough *et al.* (93) have applied the method successfully to *Salmonella*. A photographic method for locating mutants by the plating and layering method has been described by Meyersburg *et al.* (94). Auxanographic techniques have been applied extensively for identifications of nutritional requirements of mutants of *Aspergillus* by Pontecorvo (95) and *Ustilago* by Perkins (96), while Davis (91) has utilized the same principle for demonstrating the accumulation of biochemical intermediates by *E. coli* mutants.

Aliphatic amino acids.—Lewis (97) describes a group of *Neurospora* mutants that require any one of the following acids for growth: glutamic, aspartic, succinic, fumaric, malic, α-ketoglutaric, acetic. Lewis suggests an interpretation based on the assumption of a modified citric acid cycle in *Neurospora*, but it is also necessary to assume that the cycle can be blocked at at least one point without producing a lethal effect. Investigations of Fincham (98) on *Neurospora* mutants that require any one of 13 different amino acids (of which glutamate, aspartate, alanine, and ornithine are the most active) indicate that these mutants are deficient in the ability to aminate keto acids. The results also suggest that an extensive series of transamination reactions occurs in *Neurospora*. Adelberg, Bonner & Tatum (99) and Adelberg (100) have provided evidence that α,β-dihydroxy-β-methylvaleric acid is a metabolic precursor of isoleucine in *Neurospora* and *E. coli*. The substance is accumulated by certain mutants of both organisms. A

scheme relating isoleucine and threonine biosyntheses has been proposed (100) based on studies of the mutants and on demonstration of the incorporation of C^{14}-labeled acetate into isoleucine (101). The evidence is consistent with the postulate that acetate adds by the methylene carbon atom to α-hydroxy-β-ketobutyric acid followed by reduction to give the dihydroxy acid that was isolated. The entire system is evidently related through homoserine and threonine to the biosynthesis of the S-amino acids as demonstrated by Teas *et al.* (102) and Horowitz (103) for *Neurospora* and by Teas (104) for *Bacillus subtilis*. Here, homoserine was shown to be a precursor of both threonine and cystathionine, the latter giving rise eventually to methionine.

Aromatic amino acids.—Considerable progress has been reported in the past two years on the biosynthesis and interrelations of the aromatic amino acids and the vitamins nicotinic acid and *p*-aminobenzoic acid (PAB). Mutants of *Neurospora*, *Aspergillus*, *Drosophila*, and bacteria have been used for the most part. Figure 1 summarizes the present status of the problem although there is some disagreement among various workers as to the details.

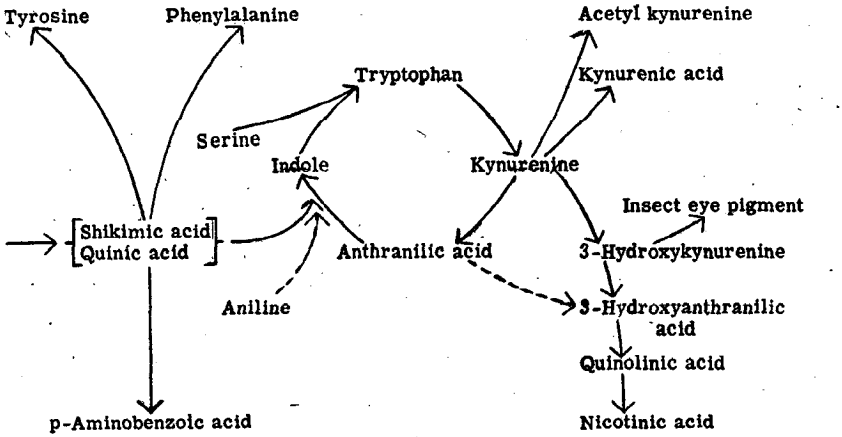

FIG. 1. Interrelations of aromatic amino acids, based on studies of several species. [Recent evidence provided by Knox & Mehler (232) and by Amano and co-workers (233) indicates that o-N-formylkynurenine is an intermediate in this series between tryptophan and kynurenine.]

A common origin for the aromatic substances shown is indicated in investigations on mutants of *E. coli* [Davis (91)] and *Neurospora* [Gordon *et al.* (105), Tatum (76) and personal communication (238)]. The positions of shikimic and quinic acids are not clear, however, since they are not interchangeable for all of the mutants. The evidence for a cycle in the metabolism of tryptophan, presented by Haskins & Mitchell (106), has been questioned by Bonner & Partridge (107) on the basis of experiments in which N^{15}-labeled indole gave rise to N^{15}-labeled quinolinic acid and "no appreciable" labeled anthranilic acid. Since the mutant used is known to accumulate considerable quantities of anthranilic acid [Tatum *et al.* (108)] which would dilute any

isotopic anthranilic acid formed, the criticism is not convincing. The possible relation of the cycle to the accumulation of polyphosphate by one of the *Neurospora* mutants (109) is worth noting.

The following intermediates shown in Figure 1 have been identified and isolated from various mutants: anthranilic acid (108), acetylkynurenine [Yanofsky & Bonner (110)], kynurenic acid [Haskins (234)], 3-hydroxyanthranilic acid [Mitchell & Nyc (111, 112), Bonner (113)], and quinolinic acid [Bonner & Yanofsky (114), Henderson (115)]. In addition, the isolation of 3-hydroxykynurenine from *Calliphora* larvae has been achieved by Butenandt *et al.* (116). Its biological activity in *Neurospora*, postulated earlier (111), was confirmed (106). More recently, Hirata *et al.* have reported the isolation of this substance from silkworms (117).

In view of the low activity of quinolinic acid, it is not certain whether the substance is an intermediate or a by-product. Bonner & Partridge (107) and Leifer *et al.* (118) show that the nitrogen atoms of indole and anthranilic acid are carried through, at least in part, to quinolinic acid and nicotinic acid in *Neurospora*. Nyc *et al.* (119) show that the carboxyl carbon of anthranilic acid is not incorporated into tryptophan and nicotinic acid in *Neurospora* but is lost as carbon dioxide. This is in line with the observation of Fries (120) that a mutant of *Lentinus* uses aniline in place of anthranilic acid. The demonstration by Heidelberger (121) that the ring carbon of tryptophan appears as the carboxyl of nicotinic acid in the rat, together with other evidence, indicates a reaction series in the rat similar to that of *Neurospora*, where genetic control can be easily demonstrated.

Pontecorvo (122) has described a mutant of *Aspergillus* that utilizes anthranilic acid, 3-hydroxyanthranilic acid, or nicotinic acid, but not indole, tryptophan, or kynurenine. An alternative metabolic path in this organism is indicated.

In *Drosophila*, it is now known that 3-hydroxykynurenine is the cn^+ substance (116). Previous studies had shown kynurenine to be the v^+ substance. Green (123) disputes the conclusion of Caspari (124) that vermilion mutants contain protein with an abnormal tryptophan content. Green finds accumulation of nonprotein tryptophan in the mutants, but no difference between mutants and wild type with respect to protein tryptophan.

Basic amino acids.—Pontecorvo (125) has reported on a mutant of *Aspergillus* that will utilize proline, ornithine, or arginine, but not citrulline. On the other hand, in accord with earlier findings in *Neurospora* (126), Volcani & Snell (127) found citrulline to be an intermediate in arginine biosynthesis in lactobacilli. The series of reactions may simply be different in the organism used, but also, there is a possibility that citrulline may not be an intermediate itself but may exist in equilibrium with an intermediate in the organisms that are able to use it. Fincham (98) has reported that α-amino-δ-hydroxyvaleric acid acts as a common precursor of ornithine and proline in *Neurospora*. A complex interrelation was observed in *Neurospora* between arginine, lysine, and pyrimidine utilization (128).

Following the discovery by Borsook *et al.* (129) that liver slices convert

lysine to aminoadipic acid, Mitchell & Houlahan (130) reported that the latter substance acts as a precursor to lysine for *Neurospora*. Subsequently, Good *et al.* (131) provided evidence that α-amino-ε-hydroxycaproic acid can act as an intermediate between aminoadipic acid and lysine. Several other steps are indicated by the number of genetic types of lysine-requiring mutants. α-Keto acids corresponding to α-aminoadipic acid, α-amino-ε-hydroxycaproic acid, and lysine do not support growth of any of the mutants under cultural conditions utilized. However, Bergström & Rottenberg (132) reported that a mutant of *Ophiostoma* would utilize lysine, α-aminoadipic acid, or α-ketoadipic acid.

Some *Neurospora* mutants requiring histidine (84) are inhibited by certain amino acid mixtures (a basic amino acid plus any of several other amino acids), and although they occur at a fairly high frequency, they were not found until selection was made using histidine alone as a growth supplement [Mitchell & Mitchell (235)].

Vitamins.—Among the B vitamins other than those already mentioned, Wagner & Guirard (133) and Wagner (134) have reported *in vitro* synthesis of pantothenic acid from β-alanine and pantoyl-lactone by preparation from wild type *Neurospora*. Strehler (135) investigated an interesting case of a *Neurospora* mutant requiring methionine or PAB. Aniline, *m*- or *p*-toluidine and *p*-nitrobenzamide will also support growth while homocysteine will not. It was assumed that the mutation in the strain limits PAB synthesis with a resulting loss in ability to methylate homocysteine. An apparently related case has been described by Davis & Mingioli (136) in which a mutant of *E. coli* was found to utilize vitamin B_{12} or methionine, but not thymidine. The mutant has been used for bioassay of vitamin B_{12}.

Nucleic acids.—Although many mutants of various organisms having requirements for purines, pyrimidines, or the corresponding nucleosides have been reported, these mutants have produced relatively little new information on the mechanism of biosynthesis. Mitchell *et al.* (137) reported accumulation of large quantities of orotic acid by a *Neurospora* mutant. Although this finding is consistent with the earlier evidence indicating that the carbon chain of uridine arises from oxaloacetic acid, orotic acid is considered to be a by-product rather than an intermediate. Guthrie (138) has shown that a purine-requiring mutant of *E. coli* will utilize adenine, guanine, xanthine, or hypoxanthine. RNA, DNA, uric acid, histidine, and 4-aminoimidazole-5-carboxamide were not utilized. The last substance is known to be produced by *E. coli* (139, 140), and the postulate of its being a purine precursor has received confirmation by the finding of Fries (141) that certain purine mutants of *Ophiostoma* can utilize it for growth. Although 4-aminoimidazole-5-carboxamide is quite evidently chemically related to substances giving rise to the purple pigment in adenineless mutants of *Neurospora* (142), its growth-supporting capacity for *Neurospora* mutants is questionable because of rapid decomposition in the medium. In this connection, Mitchell & Mitchell (143) have presented evidence that the purple adenine mutant of *Neurospora* is sufficiently inhibited by its own products

to provide a basis for selection of double adenine mutants in which the new spontaneous mutations prevent pigment formation and also accumulation of the inhibitory products. Fries (144) has demonstrated that guanine mutants of *Ophiostoma* are inhibited by adenine or hypoxanthine, and thus, selection for such mutants must be carried out on a medium containing an excess of guanine over the other purines. Loring & Fairley (145) provide evidence that a *Neurospora* mutant will use guanine in the presence of adenine, and thus, adenine is probably a precursor of guanine in this organism.

In connection with the above discussion of inheritance of biochemical capacities with respect to complex nitrogenous compounds, two papers on mutants concerned with nitrate reduction and nitrogen fixation have appeared recently (146, 147). In the former case, de la Haba suggests that reduction from nitrate occurs after formation of a linkage to carbon, but conclusive evidence is lacking.

Fatty acids.—Lein & Lein (148, 149) investigated two types of *Neurospora* mutants that require fatty acids. One type uses acetate and a considerable list of other saturated fatty acids, excepting palmitic and stearic acids, while the other type uses only oleic, linoleic, and linolenic acids. Independent pathways of synthesis of saturated and unsaturated fatty acid may be inferred. An acetate-requiring mutant of *Azotobacter* has been described (150).

Pigments.—Investigations of Kohler *et al.* (151), Zechmeister & Went (152), and Lincoln & Porter (153) have contributed considerably toward an understanding of inheritance of carotenoid pigments in tomatoes. It is apparent that genes R and T influence the conversion of phytofluene and other colorless polyenes to various isomers of lycopene, and in conjunction with gene B, to β-carotene. Zechmeister & Went postulated that genes R and T are representative of two fundamentally different types of gene functions, one, in essence, regulating the formation of lycopene or an immediate precursor and the other determining isomeric structure. Haxo (154) and Haxo & Zechmeister (155) have reported on the existence of at least eight carotenoid pigments in *Neurospora*. Lycopene and β-carotene are included, but investigations of albino and partial albino mutants in this organism have not been reported.

Granick (156) has summarized some recent findings on chlorophyll biosynthesis obtained from investigations of x-ray induced mutants of *Chlorella vulgaris*. The following sequence of reactions was suggested: glycine plus acetate→protoporphyrin→magnesium protoporphyrin→magnesium vinylprotoporphyrin→magnesium vinylpheoporphyrin phytyl ester→chlorophyll a. A number of other steps with many other intermediates is to be expected. A possible similarity in the origin of iron prophyrins is pointed out.

A number of papers have appeared concerning complexities in the genetics and biosynthesis of melanin formation. Lerner & Fitzpatrick (157) summarized the field in a review on melanin formation in mammals. Wright & Braddock (158) and Wright (159) described useful analytical techniques and their applications to guinea pigs. The melanins are divided into eumela-

nins (dark) and phaeomelanins (orange-yellow). The first type is further divided into sepia and brown, and each of these into two subgroups, while the phaeomelanins are divided into three principle sub-groups. Color intensity in each of these subgroups is modified by the C series of alleles as well as by other less well-known modifiers. Russell & Russell (160) have correlated tyrosinase activity with genetic constitution and coat color in the mouse. Markert (161) has described a great variance in pigmentation correlated with tyrosinase activity among mutants of the fungus *Glomerella*. Russell (162, 163) has reported on some interesting histological aspects of the melanin problem in the mouse. Size, nature, clumping, and degree of pigmentation are considered highly significant in phenotypic expressions. These experiments provide a more precise background for investigations of the complex and still obscure chemical differences in the melanins.

In another complex pigmentation problem, that of the plant anthocyanins and related substances, important contributions of Laughnan (164, 165) have indicated that the biochemical relations and interconversions of these substances are more obscure than indicated by some previous workers.

Miscellaneous phenomena.—The phenomenon of drug resistance and drug dependence has become commonplace among mutants of microorganisms. Demerec (166), Emerson (167), Fredericq (168), Newcombe & Hawirko (169), Clapper & Heatherman (170), and Foley & Schwachman (171) have described many cases. Similar principles are apparently involved in the heritable resistance of house flies to benzene hexachloride (172) and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane (DDT) (173). These problems are clearly of great medical and economic importance as well as genetic significance.

Heritable differences in vitamin requirements of mice have been reported by Elson (174) and Koller (175). These findings partly support the conclusions of Williams *et al.* (176) concerning the genetic basis for the etiology of alcoholism and its relation to vitamin requirements.

Genes and gene products.—The apparent one-to-one correlation between mutation of single genes and the loss in competence to carry out specific chemical reactions in *Neurospora* [see Beadle (177)] has received attention in recent reviews [Horowitz (79), Mitchell (80)]. The genes and the processes by which genes exert their control of metabolism still lack definition in biochemical terms, but it remains a reasonable hypothesis that these processes result in the production of macromolecules having specific functions as catalytic or structural substances. Recent experimental work with *Neurospora* suggests that even if a particular enzyme is a primary gene product, many biochemical reactions besides that catalyzed by the enzyme may be affected secondarily as a result of mutation of the single gene that gives rise to the enzyme. Indeed, the primary effect of such a mutation may not be the one observed by the use of limited biological or chemical criteria.

Wagner & Guirard (133) and Wagner (134) have described experiments correlating a gene-enzyme-chemical reaction in *Neurospora*. It was found that intact nongrowing mycelial pads of wild type are capable of synthe-

sizing pantothenic acid from β-alanine and pantoyl-lactone, while synthesis was not observed using pads from a pantothenic acid-requiring mutant that will not utilize a mixture of the two fragments for growth. It was subsequently shown that acetone-dried, well-washed residues from both mutant and wild type strains were capable of promoting pantothenic acid synthesis. Such preparations from x-ray and ultraviolet induced mutants, in which the same locus is apparently involved, showed different temperature coefficients of enzymatic activity than did wild type preparations. However, there has been no other demonstration of specific chemical or physical differences in the enzymes involved. Another investigation with *Neurospora* concerns a mutant that requires tryptophan but will not utilize indole plus serine (178). Although wild type and other mutants contain an enzyme for coupling these two substances, experiments failed to detect such a catalyst in cell free preparations from the mutant. The report by Gordon & Mitchell (179) that this mutant contains an altered enzyme which is subject to inhibitions by normal cell constituents has not been confirmed in a reinvestigation of the problem [Mitchell & Gordon (236)]. No enzyme activity was demonstrable in preparations from the mutant using analytical methods that will detect 0.2 per cent of the activity obtained from wild type and other mutants of *Neurospora*.

The complexities of inheritance of mammalian coat colors has already been mentioned in another connection. In a similar vein, Markert (161) has shown that in the fungus *Glomerella*, at least six different genes markedly influenced pigmentation and the amount of tyrosinase actually found in crude, cell-free enzyme preparations from the mold. It seems unlikely that all of the mutations involve a change of a gene directly responsible for tyrosinase specificity. Similarly, secondary effects may account for a considerable part of the antigenic differences found by Fox (180) in immunological work with wild type and two mutant types of *Drosophila*. In other work with *Drosophila*, Villee (181) has correlated respiratory quotients of particular imaginal discs with the phenotypic expression of the vestigial gene. It would seem that the primary effect of the mutation may or may not concern respiration directly. The same can be said of the results of the investigations of duBuy *et al.* (182) on the mitochondria of normal and mutant strains of *Nicotiana* and *Lonicera*.

Bender *et al.* (183) have found that *Neurospora* produces a soluble L-amino acid oxidase when grown in low-biotin media. The existence of strain differences in the expression of the phenomenon are reported. Considerable effort has been directed toward problems of inheritance of the potentiality for adaptive enzyme production. Klein & Doudoroff (184) reported an interesting case in which mutation in *Pseudomonas* apparently resulted in the gain of capacity to produce hexokinase adaptively. The relation of adaptation to genes has been discussed extensively by Monod (185, 186). He has concluded that a substrate initiating adaptation acts upon a gene product and not directly upon a gene. The experimental evidence was derived from investigations of *E. coli* strains adaptive and nonadaptive with respect to

lactase and amylomaltase. Lederberg (187) has shown evidence for participation of seven independent genes in influencing the capacity of this organism to produce lactase, while Bonner (82) reports that two and possibly three different gene mutations will prevent adaptation to lactose utilization and lactase formation in *Neurospora*. Lindegren & Lindegren (188) have described somewhat similar cases in yeast involving adaptive fermentations of several different sugars. A multiplicity of genes controlling competence for adaptation is indicated.

The most outstanding analysis of differences existing between two substances that are possibly primary products of gene action has been described by Pauling *et al.* (189) and Wells & Itano (190). It has been demonstrated that carbonmonoxyhemoglobin from sickle cell anemia erythrocytes differs in electrophoretic mobility from normal carbonmonoxyhemoglobin, while both types are found, in somewhat variable proportions, in sicklemia. Recent work of Neel (191) on the inheritance of the sickling character is in accord with the assumption that the gene responsible is in the homozygous condition in individuals with sickle cell anemia and in the heterozygous condition in sicklemia. In a further analysis of the carbonmonoxyhemoglobins (189), evidence was presented to show that the differences lie in the globins or in the porphyrin globin combinations. A consideration of isoelectric points indicates an excess of two to four positive charges per molecule in sickle cell anemia hemoglobin as compared to normal hemoglobin.

Non-Mendelian Inheritance

The period under review has brought forth much new work on traits showing non-Mendelian inheritance. The chief question is whether non-Mendelian inheritance necessarily implies the existence of gene-like particles in the cytoplasm (plasmagenes). This problem is of fundamental importance for biology, and its solution will mark a great advance.

The gene theory, now 35 years old, has made possible the greatest unification of the biological sciences yet attained, bringing together the diverse phenomena of heredity, evolution, and biochemistry into a consistent and satisfying general account. Despite its wide sweep, the gene theory is silent concerning the mechanism of embryonic development, characterized by the orderly differentiation of genetically identical cells. Some biologists have therefore suspected that the cytoplasm, as well as the nucleus, may contain genetic particles, a suspicion which has received sporadic support as, from time to time, new instances of non-Mendelian inheritance have been discovered. The chloroplastids, for example, are normal cytoplasmic constituents which are generally believed to be endowed with the gene-like properties of self-duplication and mutability [Rhoades (192, 193)]. On the other hand, the "killer" character in *Paramecium* and the carbon dioxide-sensitivity symptom in *Drosophila* also involve self-multiplying, mutable (194, 195) cytoplasmic factors, but being neither essential nor even usual cellular constituents, these factors cannot be distinguished from parasitic infections.

It would seem that plasmagenes can explain how cells remain different,

but not how they become different. To account for the latter, it is necessary to go back to the localization of materials in the egg. Given such a predisposition, which has been repeatedly demonstrated by embryologists, it is possible that development may eventually be accounted for without the assumption of plasmagenes. Delbrück (196) has pointed out some of the possibilities for differentiation of steady state systems under the influence of transitory fluctuations in the rate of supply one of of the components. The stability of Delbrück's model depends not on self-duplicating materials, but on relational aspects within the system.

In view of the current involvement of genetics in politics, it is worth noting that the plasmagene idea is not to be construed as an attack on Mendelian genetics. Sonneborn (197), a leading proponent of the plasmagene, has taken pains to make this clear.

Paramecium.—A detailed description of kappa, the cytoplasmic determinant of the "killer" trait in *Paramecium aurelia* is now available. Preer (198) has shown that it is a microscopically visible body, probably containing DNA and stained by the Feulgen and Giemsa stains. The stained particles are 0.2 to 0.8 μ in diameter, placing kappa in the size range of the rickettsiae. Strong killers contain 400 to 1,300 kappa particles per cell, whereas sensitive animals contain few or none. Under equilibrium conditions, the multiplication rate of kappa equals that of its host, but it may be made greater or less than this by changing conditions (199). Kappa is mutable [Dippell (194)]. It can be eliminated from killers by chloramphenicol [Brown (200)].

"Killer" lines of *Paramecium* secrete a substance (paramecin) into the medium which kills sensitive paramecia. Austin (201) presents evidence that a single particle of paramecin is sufficient to kill a sensitive animal. Van Wagtendonk (202) has continued his investigation of the chemistry of paramecin with a report on the inactivation of the substance by enzymes. Increases over the spontaneous inactivation rate were obtained by incubating crude paramecin preparations with pepsin, chymotrypsin, and desoxyribonuclease. Van Wagtendonk tentatively suggests that paramecin is a desoxyribonucleoprotein. The author's data show an apparent stabilizing effect of cysteine on paramecin, suggesting that a reducing group may be necessary for activity.

A new example of cytoplasmic inheritance in *P. aurelia* variety 4 has been found by Sonneborn (203 to 206). When cultured under standard conditions, a given line of stocks 29 or 51 can be characterized as belonging to one of nine serological types. Dilute antisera against a line immobilize animals belonging to the homologous serotype. Exposure to immobilizing antiserum or to changes in temperature or food supply induces transformation into other serotypes which are again stable when returned to standard conditions. Directed transformations can be accomplished by adjustment of transforming conditions. Since individuals of a given stock are genetically identical, it is thus possible to have diversity of serotypes with uniformity of genes. In crosses between individuals of the same stock, the inheritance of the serotype follows the cytoplasm. That the genes are the ultimate de-

terminants of the antigenic potentialities, however, is shown by crosses between stocks. From these, it appears that the genes determine (*a*) the specificity of the antigens and (*b*) the nature of the response to a given transforming stimulus. These results are in line with established genetic and developmental principles in other organisms. The point of special interest is that the *Paramecium* material, in which somatic and gametic functions are combined in the same cell, offers an opportunity for the analysis of the mechanisms underlying the persistence of induced changes and may thus throw light on the major problems of development.

Yeast.—As the result of a careful study of the genetics of maltose and galactose fermentations in yeast, Winge & Roberts (207) have concluded that these characters are inherited in a normal Mendelian fashion. They indicate that the non-Mendelian segregations reported earlier by Lindegren (208) and Spiegelman *et al.* (209) resulted from misclassification of certain types as genetic nonfermenters, when actually they were fermenters with relatively long adaptation times. An additional explanation of apparent non-Mendelian results in yeast has been advanced in a recent note by the same authors (210). They find that extra divisions occur in some asci, and they point out that random degeneration of spores in such asci would lead to erroneous conclusions in genetic analyses. Mundkur & Lindegren (211, 212, 213) take issue with Winge & Roberts on the above points, stating that long-term adaptation in fact results from mutation and selection and that the inheritance of fermentation and vitamin-synthesizing abilities is often non-Mendelian in some yeast strains. Pompér & Burkholder (214), on the other hand, find no evidence for non-Mendelian inheritance in a number of biochemical mutants of yeast. In view of the unpredictable nature of the results obtained by Lindegren & Mundkur, the difficulties reported in classifying multiple-deficiency mutants (215), and the lack of confirmation by others, their conclusions are not altogether convincing.

The most credible report of non-Mendelian inheritance in yeast involves the mutation *petite colonie*, described in a series of papers from Ephrussi's laboratory (216 to 222). This mutation is probably identical with that obtained by Stier & Castor (223) some years ago. The *petite* mutation occurs spontaneously in baker's yeast at the rate of approximately 0.002 per cell generation. Treatment with 2,8-diamino-10-methylacridinium chloride (acriflavine) results in practically complete transformation of normal cultures into *petites*. Biochemically, the latter are characterized by the lack of detectable cytochrome oxidase and succinic dehydrogenase activities. Cytochrome-*c* is present. Respiration of glucose is only 8 per cent of normal, but fermentation is normal and proceeds at the same rate aerobically as anaerobically (absence of Pasteur effect). In crosses to normal and in backcrosses, non-Mendelian results were obtained. The character is lost on outcrossing, appearing among the progeny with a frequency approximating the spontaneous mutation rate. In contrast, it is quite stable through asexual divisions. These and other results suggest that self-duplicating cytoplasmic elements, present in normal and lacking in mutant cells, are involved. It is suggested

that these elements may be mitochondria. Recently, it has been found that the *petite* character can also result from mutation of a nuclear gene (224). When the gene mutant is crossed to the cytoplasmic mutant, half the progeny are normal, a result consistent with the hypothesis that the cyanide-sensitive respiratory system is controlled by a nuclear gene and a cytoplasmic factor. Although the foregoing results argue strongly in favor of a self-reproducing element in the cytoplasm, Sturtevant (237) has pointed out the similarities between this case and the position effect variegations in *Drosophila* [reviewed by Lewis (225)]. Although the mechanisms underlying position effects are themselves not well understood, they are known to have a definite chromosomal basis.

Higher animals.—Billingham & Medawar (226, 227) describe the results of skin-grafting experiments which have led them to suggest that melanin formation in the guinea pig is mediated by a self-reproducing enzyme (or enzyme precursor). When pigmented skin is grafted into a nonpigmented region, or the reverse, spreading of pigment into the nonpigmented area around the graft is observed. The authors demonstrate convincingly that the spreading is not the result of invasive cellular replacement or of ordinary enzyme or metabolite diffusion. A third possibility, that of melanophore migration, is considered to be unlikely on the basis of the evidence but has not been disproved. The most probable interpretation, in the view of Billingham & Medawar is transmission of a melanogenic element by infection from pigmented to nonpigmented cells.

Along similar lines, Niu & Twitty (228) have reported direct observations of the transformation of phagocytic cells of salamander larvae into melanophores following the ingestion of debris from degenerating melanophores. While in a sense complementary to the work of Billingham & Medawar, there is yet no proof of an increase in the total quantity of melanogenic enzymes in the transformed cell lines.

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CONTENTS